Response to Reviewer 2 Comments

We sincerely appreciated the critical and invaluable comments provided by the reviewers for our manuscript. Our point-by-point responses to all comments are given below. Revised and added contents in the manuscript are highlighted in red fonts.

Comments 1:

Quality of English Language

(x) Minor editing of English language required

Response 1:

Thank you for pointing this out. We agree with this comment. Therefore, we have thoroughly checked the English language editing and revised the contents of whole manuscript along with supplementary file. The revision parts were highlighted in red fonts. The certicate for English editing was also uploaded.

Comments 2:

One concern with the manuscript is the significant verbatim reproduction of material within the main text from other published sources especially from the paper cited above.

In the abstract, the authors need to include more details such as sample size, method of study, ethical approval etc. Currently, the abstract goes from the aim to the results. Additionally, some points are focused and highlighted that are not really discussed in the manuscript, for example, "provoking periodontal regeneration" is highlighted in the abstract but not really discussed or focused on in the manuscript. I would urge the authors to rewrite the abstract per the journal guidelines and to include relevant content related to the methods.

Response 2:

Thank you for pointing this out. We agree with this comment. As a continued research following our previous work ("*Huang AC, Ishida Y, Li K, Rintanalert D, Hatano-Sato K, Oishi S, Hosomichi J, Usumi-Fujita R, Yamaguchi H, Tsujimoto H, Sasai A, Ochi A, Watanabe H, Ono T. NF-κB Decoy ODN-Loaded Poly(Lactic-co-glycolic Acid Nanospheres Inhibit Alveolar Ridge Resorption. Int J Mol Sci. 2023 Feb 12;24(4):3699.*"), current research adopted identical reagant design as described in the manuscript and supplementary materials section but within different region and objectives. Therefore, here we have accordingly revised the *4.1. Decoy ODN Nuclear Medicine* section with below texts containing contents used to emphasize this point, the revisions can also be found in re-submitted manuscript:

> In continuation of our previous research, naked scrambled ODNs (ScD), structured as phosphorothioated double-stranded ScD with sequences 5'-TTGCCGTACCTGACTTAGCC-3' and 3'-AACGGCATGGACTGAATCGG-5', and NF-κB-targeting ODNs (NfD), structured as phosphorothioated double-stranded NfD with sequences 5'-CCTTGAAGGGATTTCCCTCC-3' and 3'-GGAACTTCCCTAAAGGGAGG-5', were used as previously described [17]. The concentrations of ScD and NfD in solutions containing ScD and NfD, as well as in PLGA-ScD and PLGA-NfD

solutions, respectively. The concentration of hydroxypropyl cellulose-H with PLGA NS and the physicochemical properties of decoy ODN-loaded PLGA NS were determined according to Huang et al. [17]. Reagents for the NfD and PLGA-NfDs were provided by AnGes Inc. (Osaka, Japan) and Hosokawa Micron Corporation (Osaka, Japan).

In the abstract section, the reviewer can also find the revised contents as shown below along with uploaded re-submitted manuscript.

> Orthodontic space closure following tooth extraction is often hindered by alveolar bone deficiency. This study investigates the therapeutic use of nuclear factor-kappa B (NF-κB) decoy oligodeoxynucleotides loaded with polylactic-co-glycolic acid nanospheres (PLGA-NfDs) to mitigate alveolar bone loss during orthodontic tooth movement (OTM) following the bilateral extraction of maxillary first molars in a controlled experiment involving 40 OTM rats model with ethics approved. The decreased tendency of the OTM distance and inclination angle with increased bone volume and improved trabecular bone structure indicated minimized alveolar bone destruction. Reverse transcription-quantitative polymerase chain reaction and histomorphometric analysis demonstrated the suppression of inflammation and bone resorption by downregulating the expression of tartrate-resistant acid phosphatase, tumor necrosis factor-α, interleukin-1β, cathepsin K, NF-κB p65, and receptor activator of NF-κB ligand while provoking periodontal regeneration by upregulating the expression of alkaline phosphatase, transforming growth factor-β1, osteopontin, and fibroblast growth factor-2. Importantly, relative gene expression over the maxillary second molar compression side in proximity to the alveolus highlighted the pharmacological effect of intra-socket PLGA-NfD administration, as evidenced by elevated osteocalcin expression, indicative of enhanced osteocytogenesis. These findings emphasize that locally administered PLGA-NfD serves as an effective inflammatory suppressor and yields periodontal regenerative responses following tooth extraction.

Regarding points focused and highlighted that are not really discussed in the manuscript, the reviewer can find not only the revised contents from abstrat text shown above (These findings emphasize that locally administered PLGA-NfD serves as an effective inflammatory suppressor and yields periodontal regenerative responses following tooth extraction.), in the main text of the manuscript, line 52, 79, 81, 215, 220, 259, 280, 283, 285 ,287, 288, 298 has also been revised to contain the revised text regarding periodontal regeneration along with the re-submitted manuscript. "Provoking periodontal regeneration" has been highlighted in the abstract and main text from the manuscript with more focused and discussed revised contents which can also be seen from the uploaded revised manuscript. The abstract has also been rewrited per the journal guidelines which has already been included with relevant content related to the methods as shown above. Please find the detailed revisions highlighted red thorughout the whole manuscript and abstract updated and uploaded.